

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup>:</b> <b>A61K 31/00, 31/425, 31/44</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 95/21608</b> <b>(43) International Publication Date:</b> 17 August 1995 (17.08.95)
<b>(21) International Application Number:</b> PCT/EP95/00441 <b>(22) International Filing Date:</b> 7 February 1995 (07.02.95)  <b>(30) Priority Data:</b> 9402624.2 10 February 1994 (10.02.94) GB 9410214.2 21 May 1994 (21.05.94) GB 9426019.7 22 December 1994 (22.12.94) GB  <b>(71) Applicant (for all designated States except US):</b> SMITHKLINE BEECHAM PLC [GB/GB]; New Horizons Court, Brentford, Middlesex TW8 9EP (GB).  <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> BUCKINGHAM, Robin, Edwin [GB/GB]; SmithKline Beecham Pharmaceuticals, The Frythe, Welwyn, Hertfordshire AL6 9AR (GB).  <b>(74) Agent:</b> RUTTER, Keith; SmithKline Beecham, Corporate Intellectual Property, SB House, Great West Road, Brentford, Middlesex TW8 9BD (GB).		<b>(81) Designated States:</b> AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> USE OF INSULIN SENSITISERS FOR TREATING RENAL DISEASES		
<b>(57) Abstract</b>  A method for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria which method comprises the administration of an effective, non-toxic amount of an insulin sensitiser to a human or non-human mammal in need thereof.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LV	Latvia	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

## Use of insulin sensitisers for treating renal diseases

This invention relates to a novel method for the treatment of renal diseases.

5       European Patent Applications, Publication Numbers: 0306228, 0008203, 0139421, 0032128, 0428312, 0489663, 0155845, 0257781, 0208420, 0177353, 0319189, 0332331, 0332332, 0528734, 0508740; International Patent Application, Publication Numbers 92/18501, 93/02079, 93/22445 and United States Patent Number 5104888, disclose certain thiazolidinedione derivatives  
10       which are disclosed as having hypoglycaemic and hypolipidaemic activity. The thiazolidinedione derivatives disclosed in these patent applications are examples of a class of hypoglycaemic agent generally referred to as 'insulin sensitisers' and hence these compounds are referred to herein as 'thiazolidinedione insulin sensitisers'.

15       Another series of compounds generally recognised as having insulin sensitiser activity are those typified by the compounds disclosed in International Patent Applications, Publication Numbers WO93/21166 and WO94/01420. These compounds are herein referred to as 'acyclic insulin sensitisers'. Other examples of acyclic insulin sensitisers are those disclosed in United States Patent  
20       Number 5232945 and International Patent Applications, Publication Numbers WO92/03425 and WO91/19702.

Examples of other insulin sensitisers are those disclosed in European Patent Application, Publication Number 0533933, Japanese Patent Application Publication Number 05271204 and United States Patent Number 5264451.

25       We have now discovered that compounds having insulin sensitiser activity can prevent hydronephrosis and proteinuria, such as albuminuria, and that they are therefore of potential use in the treatment and/or prophylaxis of renal disease, especially renal disease associated with the development of Type II diabetes including diabetic nephropathy, glomerulonephritis, glomerular sclerosis,  
30       nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease. The prophylactic action of an insulin sensitiser upon nephropathy is also indicative that an insulin sensitising agent can be expected to prevent, reverse, stabilise or retard the progression of microalbuminuria to albuminuria. This is because microalbuminuria is considered to be a predictor of future nephropathy,  
35       especially in patients with clinical evidence of pre-diabetic insulin resistance

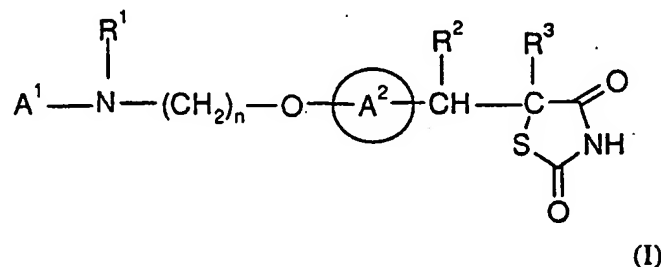
syndrome, alternatively referred to as Syndrome X.

Accordingly, the present invention provides a method for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive  
 5 nephrosclerosis and end stage renal disease, and microalbuminuria which method comprises the administration of an effective, non-toxic amount of an insulin sensitiser to a human or non-human mammal in need thereof.

Particular insulin sensitisers include thiazolidinedione insulin sensitisers.

Particular insulin sensitisers include acyclic insulin sensitisers.

10 One favoured group of insulin sensitisers are the thiazolidinedione insulin sensitisers disclosed in EP 0306228 and WO94/05659. Thus in a favoured aspect the present invention provides a method for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal  
 15 disease, and microalbuminuria which method comprises the administration of an effective non-toxic amount of a compound of formula (I):



(I)

20

or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof, and/or a pharmaceutically acceptable solvate thereof, wherein:

A<sup>1</sup> represents a substituted or unsubstituted aromatic heterocyclyl group;

R<sup>1</sup> represents a hydrogen atom, an alkyl group, an acyl group, an aralkyl group,

25 wherein the aryl moiety may be substituted or unsubstituted, or a substituted or unsubstituted aryl group;

R<sup>2</sup> and R<sup>3</sup> each represent hydrogen, or R<sup>2</sup> and R<sup>3</sup> together represent a bond;

A<sup>2</sup> represents a benzene ring having in total up to five substituents; and

30 n represents an integer in the range of from 2 to 6; to a human or non-human mammal in need thereof.

Suitable aromatic heterocyclyl groups of the compounds of formula (I) include substituted or unsubstituted, single or fused ring aromatic heterocyclyl groups comprising up to 4 hetero atoms in each ring selected from oxygen, sulphur or nitrogen.

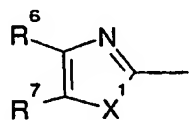
- 5 Favoured aromatic heterocyclyl groups of the compounds of formula (I) include substituted or unsubstituted single ring aromatic heterocyclyl groups having 5 to 7 ring atoms, preferably 5 or 6 ring atoms.

In particular, the aromatic heterocyclyl groups of the compounds of formula (I) comprise 1, 2 or 3 heteroatoms, especially 1 or 2, selected from oxygen, sulphur or nitrogen.

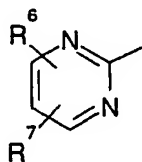
10 Suitable values for  $A^1$  when it represents a 5- membered aromatic heterocyclyl group include thiazolyl and oxazolyl, especially oxazolyl.

Suitable values for  $A^1$  when it represents a 6- membered aromatic heterocyclyl group include pyridyl or pyrimidinyl, especially pyridyl.

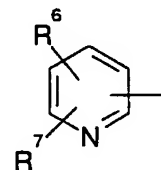
- 15 Preferably,  $A^1$  represents a moiety of formula (a), (b) or (c):



(a)



(b)

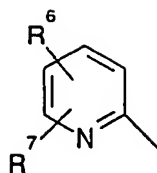


(c)

wherein:

- 20  $R^6$  and  $R^7$  each independently represents a hydrogen or halogen atom, an alkyl or alkoxy group or a substituted or unsubstituted aryl group or when  $R^6$  and  $R^7$  are each attached to adjacent carbon atoms, then  $R^6$  and  $R^7$  together with the carbon atoms to which they are attached form a benzene ring wherein each carbon atom represented by  $R^6$  and  $R^7$  together is substituted or unsubstituted; and in the moiety of formula (a)  $X^1$  represents oxygen or sulphur.

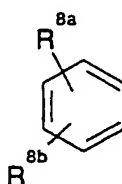
- 25 Aptly,  $A^1$  represents a moiety of the abovedefined formula (a).  
 Aptly,  $A^1$  represents a moiety of the abovedefined formula (b).  
 Aptly,  $A^1$  represents a moiety of the abovedefined formula (c).  
 A particular form of moiety (c) is a moiety (c'):



(c')

wherein  $R^6$  and  $R^7$  are as defined in relation to formula (c).

- 5 In one favoured aspect  $R^6$  and  $R^7$  together represent a moiety of formula (d):



(d)

10

wherein  $R^{8a}$  and  $R^{8b}$  each independently represent hydrogen, halogen, substituted or unsubstituted alkyl or alkoxy.

- Suitably,  $R^{8a}$  and  $R^{8b}$  each independently represent hydrogen, halogen, alkyl or alkoxy. Favourably,  $R^{8a}$  represents hydrogen. Favourably,  $R^{8b}$  represents hydrogen. Preferably,  $R^{8a}$  and  $R^{8b}$  both represent hydrogen.

- 15 In a further favoured aspect  $R^6$  and  $R^7$  each independently represent hydrogen, alkyl or a substituted or unsubstituted phenyl group and more favourably,  $R^6$  and  $R^7$  each independently represent hydrogen, alkyl or phenyl.

- 20 Preferably, for the moiety of formula (a),  $R^6$  and  $R^7$  together represent the moiety of formula (d).

Preferably, for the moieties of formula (b), (c) or (c'),  $R^6$  and  $R^7$  both represent hydrogen.

- It will be appreciated that the five substituents of  $A^2$  include three optional substituents. Suitable optional substituents for the moiety  $A^2$  include halogen, substituted or unsubstituted alkyl or alkoxy.

- 25 Further suitable, favoured and preferred values for variables  $A^2$ ,  $R^1$ ,  $R^2$ ,  $R^3$  and  $n$  are as defined in EP 0306228 and WO94/05659.

A preferred compound of formula (I) is 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione or a tautomeric form thereof

and/or a pharmaceutically acceptable salt thereof, especially a maleic acid salt thereof, and/or a pharmaceutically acceptable solvate thereof.

As indicated above, a compound of formula (I) may exist in one of several tautomeric forms, all of which are encompassed in the method of the present invention. It will be appreciated that the present invention encompasses the administration of all of the isomeric forms of the compounds of formula (I) and the pharmaceutically acceptable salts thereof, including any stereoisomeric forms thereof, whether as individual isomers or as mixtures of isomers.

Suitable substituents for any heterocyclcyl group of the compounds of formula (I) include up to 4 substituents selected from the group consisting of: alkyl, alkoxy, aryl and halogen or any two substituents on adjacent carbon atoms, together with the carbon atoms to which they are attached, may form an aryl group, preferably a benzene ring, and wherein the carbon atoms of the aryl group represented by the said two substituents may themselves be substituted or unsubstituted.

The suitable, favoured and preferred thiazolidinedione insulin sensitisers disclosed in European Patent Applications, Publication Numbers: 0306228, 0008203, 0139421, 0032128, 0428312, 0489663, 0155845, 0257781, 0208420, 0177353, 0319189, 0332331, 0332332, 0528734, 0508740; International Patent Application, Publication Numbers 92/18501, 93/02079, 93/22445 and United States Patent Number 5104888 are those compounds defined as suitable, favoured and preferred in the respective patent publications.

The suitable, favoured and preferred acyclic insulin sensitisers disclosed in International Patent Applications, Publication Numbers WO91/19702, WO92/03425, WO93/21166 and WO94/01420 and United States Patent Number 5232945 are those compounds defined as suitable, favoured and preferred in the respective patent publications.

Other suitable, favoured and preferred insulin sensitisers are the suitable, favoured and preferred compounds disclosed in European Patent Application, Publication Number 0533933, International Patent Application, Publication Number WO 93/02079, Japanese Patent Application Publication Number 05271204 and United States Patent Number 5264451.

Also specifically included in the method of the invention are the specific examples disclosed in the above mentioned patent applications.

When used herein the term 'insulin sensitiser' relates to compounds which

increase the biological response to insulin. In addition, based upon the observed effects in appropriate test animals such as Zucker fatty (fa/fa) rats, insulin sensitisers are indicated to lower elevated fasting plasma insulin concentrations and improve glycaemic control.

- 5           When used herein the term 'aryl' includes phenyl and naphthyl optionally substituted with up to five, preferably up to three, groups selected from halogen, alkyl, phenyl, alkoxy, haloalkyl, hydroxy, amino, nitro, carboxy, alkoxycarbonyl, alkoxycarbonylalkyl, alkylcarbonyloxy, or alkylcarbonyl groups.

- 10           When used herein the term 'halogen' refers to fluorine, chlorine, bromine and iodine; preferably chlorine.

          When used herein the terms 'alkyl' and 'alkoxy' relate to groups having straight or branched carbon chains, containing up to 12 carbon atoms.

          When used herein the term 'acyl' includes alkylcarbonyl groups.

- 15           Suitable alkyl groups are C<sub>1-12</sub> alkyl groups, especially C<sub>1-6</sub> alkyl groups e.g. methyl, ethyl, n-propyl, iso-propyl, n-butyl, isobutyl or tert-butyl groups.

          Suitable substituents for any alkyl group include those indicated above in relation to the term "aryl".

          Suitable pharmaceutically acceptable salts include salts of carboxy groups and acid addition salts.

- 20           Suitable pharmaceutically acceptable salts of carboxy groups include metal salts, such as for example aluminium, alkali metal salts such as lithium, sodium or potassium, alkaline earth metal salts such as calcium or magnesium and ammonium or substituted ammonium salts, for example those with lower alkylamines such as triethylamine, hydroxy alkylamines such as  
25   2-hydroxyethylamine, bis-(2-hydroxyethyl)-amine or tri-(2-hydroxyethyl)-amine, cycloalkylamines such as bicyclohexylamine, or with procaine, dibenzylpiperidine, N-benzyl-b-phenethylamine, dehydroabietylamine, N,N'-bisdehydroabietylamine, glucamine, N-methylglucamine or bases of the pyridine type such as pyridine, collidine, quinine or quinoline.

- 30           Suitable acid addition salts include pharmaceutically acceptable inorganic salts such as the sulphate, nitrate, phosphate, borate, hydrochloride and hydrobromide and pharmaceutically acceptable organic acid addition salts such as acetate, tartrate, maleate, citrate, succinate, benzoate, ascorbate, methane-sulphonate, α-keto glutarate and α-glycerophosphate, especially the maleate salt.

- 35           Suitable pharmaceutically acceptable solvates include hydrates.



The active compounds disclosed in the above mentioned patent publications, and referred to herein as insulin sensitisers, including the specific examples disclosed therein, are conveniently prepared according to the methods disclosed in the said patent publications: Thus a compound of formula (I), or the  
5 tautomeric form thereof, and/or a pharmaceutically acceptable salt thereof, and/or a pharmaceutically acceptable solvate thereof, may be prepared using the processes described in EP 0306228 and WO94/05659.

The salts and/or solvates of the compounds of formula (I) may be prepared and isolated according to conventional procedures for example sodium  
10 salts may be prepared by using sodium methoxide in methanol.

The present invention also provides an insulin sensitiser, such as a compound of formula (I), or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof, for use in the treatment of and/or prophylaxis of renal diseases including diabetic  
15 nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria.

The present invention also provides an insulin sensitiser, such as a compound of formula (I), or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof, for  
20 use in the manufacture of a medicament for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria.

In the above mentioned treatment and or prophylaxis the insulin sensitiser  
25 such as a compound of formula (I), or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof, may be administered per se or, preferably, as a pharmaceutical composition also comprising a pharmaceutically acceptable carrier.

Accordingly, the present invention also provides a pharmaceutical  
30 composition for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease, and microalbuminuria which composition comprises an insulin sensitiser, such as a compound of the formula (I), or a tautomeric form thereof, or a pharmaceutically  
35 acceptable salt thereof, or a pharmaceutically acceptable solvate thereof, and a

pharmaceutically acceptable carrier therefor.

As used herein the term 'pharmaceutically acceptable' embraces compounds, compositions and ingredients for both human and veterinary use: for example the term 'pharmaceutically acceptable salt' embraces a veterinarily  
5 acceptable salt.

The composition may, if desired, be in the form of a pack accompanied by written or printed instructions for use.

Usually the pharmaceutical compositions of the present invention will be adapted for oral administration, although compositions for administration by  
10 other routes, such as by injection and percutaneous absorption are also envisaged.

Particularly suitable compositions for oral administration are unit dosage forms such as tablets and capsules. Other fixed unit dosage forms, such as powders presented in sachets, may also be used.

In accordance with conventional pharmaceutical practice the carrier may  
15 comprise a diluent, filler, disintegrant, wetting agent, lubricant, colourant, flavourant or other conventional adjuvant.

Typical carriers include, for example, microcrystalline cellulose, starch, sodium starch glycollate, polyvinylpyrrolidone, polyvinylpolypyrrolidone, magnesium stearate, sodium lauryl sulphate or sucrose.

20 Most suitably the composition will be formulated in unit dose form. Such unit dose will normally contain an amount of the active ingredient in the range of from 0.1 to 1000 mg, more usually 0.1 to 500 mg, and more especially 0.1 to 250 mg.

Conveniently, the active ingredient may be administered as a  
25 pharmaceutical composition hereinbefore defined, and this forms a particular aspect of the present invention.

In the above mentioned treatments an insulin sensitiser, such as a compound of the formula (I), or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable  
30 solvate thereof, may be taken in doses, such as those described above, one to six times a day in a manner such that the total daily dose for a 70 kg adult will generally be in the range of from 0.1 to 6000 mg, and more usually about 1 to 1500 mg, generally about 0.5 to 10 mg. That is in the range of from  $1.429 \times 10^{-3}$  to 85.714 mg/kg/day, more usually about  $1.429 \times 10^{-2}$  to 21.429 mg/kg/day,  
35 generally about  $7.143 \times 10^{-3}$  to 0.1429 mg/kg/day.

The following Examples illustrate the invention but do not limit it in any way.

### Example 1: Studies Into The Effects Of The Test Compound Upon Renal Pathology

Obese Zucker rats are known to develop chronic nephropathy in addition  
5 to hyperlipidaemia, hyperinsulinaemia and peripheral insulin resistance (Kasiske  
*et al* 1985).

5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-  
dione (herein after referred to as 'test compound') was administered to 2-3 month  
old obese Zucker fa/fa rats by dietary administration to provide a daily dose over  
10 a period of 3 months ranging from 2.0 - 7.0  $\mu$ mole/kg body weight. A group of  
age-matched obese Zucker fa/fa rats were given the same diet without the  
addition of the test compound, as was a group of lean Fa/? rats. At the end of the  
study, all of the control Zucker fa/fa rats had chronic nephropathy which involved  
dilated tubules, atrophied or hyperplastic tubular epithelial cells, thickened  
15 tubular basement membranes, segmented glomeruli or global glomerulosclerosis.

In the corresponding lean animals, 3/15 animals, a minimal degree of  
chronic nephropathy was seen.

Treatment with test compound resulted in a reduction in the incidence and  
degree of chronic nephropathy compared to the control group of Zucker fa/fa rats.

20 In the kidneys of the control Zucker fa/fa rats, mild-moderate  
hydronephrosis was seen in 4/9 animals. Characteristically this involved dilation  
of the kidney pelvis. No hydronephrosis was seen in the rats treated with test  
compound.

TABLE OF SIGNIFICANT HISTOPATHOLOGICAL  
FINDINGS IN THE KIDNEY OF FATTY ZUCKER RATS

Group/Treatment Animal Number	Hydronephrosis	Chronic Nephropathy
----------------------------------	----------------	---------------------

**Group 1**

Test Compound in diet

2	0	±
7	0	±
9	0	0
15	0	++
16	0	±
17	0	+
21	0	±
22	0	±
24	0	±
27	0	±
28	0	±

**Group 2**

Diet only

3	++	+
5	+++	+
13	0	+
14	++	++
18	0	+
23	+++	++
26	0	+
29	0	+
30	0	++

5

Severity Key

0      None seen

±	Minimal
+	Mild
++	Moderate
+++	Marked

**Example 2: Studies Into The Effect Of Test Compounds Upon Systolic Blood Pressure, Urinary Total Protein And Urinary N-Acetyl  $\beta$ -D-Glucosaminidase (NAG) Activity Measurements.**

- 5 A second study was performed in Zucker rats over a period of 9 months to investigate the longitudinal effects of the drug on systolic blood pressure and various indices of renal function, two of which are exemplified here. In one arm of the study, the drug was given in the diet (50  $\mu$ mole/kg of diet) from aged 6-7 weeks in Zucker fatty (fa/fa) rats, whilst in a second arm of the study, drug
- 10 treatment as above was delayed until proteinuria had become established after 4 months, indicative of structural damage already present in the kidneys. A third group of Zucker fatty rats and a further group of lean rats were given diet alone throughout the period of study.
- 15 After dosing with the test compound, at monthly intervals measurements were made of systolic blood pressure, urinary total protein and urinary N-acetyl  $\beta$ -D-glucosaminidase (NAG) activity.

*Measurement of Systolic Blood Pressure*

- 20 Rats were enclosed in custom-built restrainers and placed on a shelf in a warming cabinet, whose temperature was controlled at approximately 30°C. An inflatable cuff with integral pulse sensor was attached to the tail of each rat. After warming for 20-30 min the tail cuff was inflated and deflated automatically and a measurement of systolic blood pressure made using an IITC Non-Invasive Blood
- 25 Pressure Monitor. This cycle was repeated several times for each rat until stable values of blood pressure were obtained.

*Measurement of Urinary Parameters*

- Twenty-four hour urine collections, made in metabolism cages, were aliquoted
- 30 and frozen at -70°C until required for assay.

*(i) Urinary Protein*

- Urinary protein concentration was measured using the Bio-Rad protein assay as modified for use in a 96-well microtitre plate. The assay is a dye-binding assay
- 35 based on the differential colour change of a dye in response to various

concentrations of protein (Bradford, Anal. Biochem., 72, 248, 1976). After a period of incubation with Dye Reagent, diluted urine samples are read at an optical density of 595 nm using a Molecular Devices multiplate reader.

5   (ii) *Urinary NAG Activity*

NAG activity was assayed using a reagent kit on a Hitachi 717 analyser (both supplied by Boehringer Mannheim UK, Lewes). Enzyme activity was measured by monitoring the rate of chlorophenol (570 nm) released from the substrate chlorophenol red- $\beta$ -D-glucosaminide.

10

*Results and Statistics*

In the tables below, results are given as mean values for the group  $\pm$  the standard error of the mean. Results have been analysed by one way ANOVA and significant differences from the Zucker fatty rat control group have been indicated by an asterisk.

15

*Conclusion*

The results of Example 2 demonstrate that treatment of Zucker fatty (fa/fa) rats from the age of 6-7 weeks, for a period of 9 months, with BRL 49653 given via the diet, prevented the development of hypertension and markedly reduced both the elevation of urinary NAG activity and the rate of development of proteinuria. When drug treatment was commenced after proteinuria had become established, both systolic blood pressure and urinary NAG activity were prevented from rising further and again there was a marked reduction of the rate of increase in the urinary protein concentration.

20  
25



**Effects of starting treatment with Test Compound prior to the  
development of renal complications.**

5

**SYSTOLIC BLOOD PRESSURE (mm Hg)**

Treatment Duration (months)	Zucker fatty rats Test Compound (50 µmol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
0	102 ± 3	106 ± 3	123 ± 3*
1	110 ± 3 *	120 ± 4	128 ± 3
2	122 ± 4 *	137 ± 4	132 ± 4
3	122 ± 4 *	142 ± 6	134 ± 3
4	126 ± 6 *	146 ± 4	132 ± 4*
5	131 ± 4 *	157 ± 6	138 ± 4*
6	128 ± 3 *	150 ± 6	133 ± 2*
7	125 ± 6*	157 ± 6	128 ± 6*
8	133 ± 5*	155 ± 4	130 ± 3*
9	143 ± 4*	164 ± 4	136 ± 3*

10

**Effects of starting treatment with Test Compound prior to the  
development of renal complications**

15

**URINARY PROTEIN CONCENTRATION (µg/hour)**

Treatment Duration (months)	Zucker fatty rats Test Compound (50 µmol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
0	226 ± 15 *	287 ± 17	198 ± 19*
1	373 ± 22	355 ± 18	321 ± 23*
2	402 ± 22 *	509 ± 28	396 ± 20*
3	221 ± 32 *	874 ± 102	393 ± 25*
4	376 ± 54 *	3965 ± 731	613 ± 52*
5	1573 ± 105 *	5823 ± 809	1395 ± 88*
6	1585 ± 147 *	8946 ± 1171	1192 ± 92*
7	2124 ± 293 *	12052 ± 1535	1176 ± 96*
8	2664 ± 370*	14534 ± 1540	1409 ± 92*
9	3152 ± 515*	16182 ± 1581	1373 ± 113*

Effects of starting treatment with Test Compound prior to the development of renal complications.

5

### URINARY N-ACETYL - $\beta$ - D-GLUCOSAMINIDASE ACTIVITY (mU/hour)

10

Treatment Duration (months)	Zucker fatty rats Test Compound (50 $\mu$ mol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
0	5.7 $\pm$ 0.4	5.9 $\pm$ 0.3	4.4 $\pm$ 0.3*
1	7.5 $\pm$ 0.7	9.1 $\pm$ 0.8	5.1 $\pm$ 0.5*
2	3.8 $\pm$ 1.1 *	8.2 $\pm$ 1.5	6.5 $\pm$ 0.3
3	4.5 $\pm$ 0.9 *	8.2 $\pm$ 1.0	5.2 $\pm$ 0.4*
4	5.1 $\pm$ 1.2 *	9.3 $\pm$ 0.5	6.9 $\pm$ 0.5*
5	6.5 $\pm$ 0.7 *	10.7 $\pm$ 0.4	7.6 $\pm$ 0.7*
6	6.5 $\pm$ 1.1 *	10.8 $\pm$ 1.7	7.4 $\pm$ 0.5
7	8.9 $\pm$ 0.8*	11.7 $\pm$ 0.7	7.1 $\pm$ 0.5*
8	6.2 $\pm$ 1.2*	11.8 $\pm$ 1.3	7.4 $\pm$ 0.5*
9	9.1 $\pm$ 1.2*	13.4 $\pm$ 0.8	7.7 $\pm$ 0.6*

Effects of starting treatment with Test Compound once renal complications have become established.

15

### SYSTOLIC BLOOD PRESSURE (mm Hg)

Treatment Duration (months)	Zucker fatty rats Test Compound (50 $\mu$ mol/kg of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
-4	106 $\pm$ 3	106 $\pm$ 3	123 $\pm$ 3*
-3	123 $\pm$ 5	120 $\pm$ 4	128 $\pm$ 3
-2	138 $\pm$ 7	137 $\pm$ 4	132 $\pm$ 4
-1	141 $\pm$ 6	142 $\pm$ 6	134 $\pm$ 3
0	146 $\pm$ 4	146 $\pm$ 4	132 $\pm$ 4*
1	140 $\pm$ 5*	157 $\pm$ 6	138 $\pm$ 4*
2	134 $\pm$ 3*	150 $\pm$ 6	133 $\pm$ 2*
3	144 $\pm$ 3*	157 $\pm$ 6	128 $\pm$ 6*
4	139 $\pm$ 3*	155 $\pm$ 4	130 $\pm$ 3*
5	146 $\pm$ 6*	164 $\pm$ 4	136 $\pm$ 3*

Effects of starting treatment with Test Compound once renal complications have become established.

5

### URINARY PROTEIN CONCENTRATION ( $\mu\text{g}/\text{hour}$ )

Treatment Duration (months)	Zucker fatty rats Test Compound (50 $\mu\text{mol}/\text{kg}$ of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
-4	220 $\pm$ 13	287 $\pm$ 17	198 $\pm$ 19*
-3	347 $\pm$ 32	355 $\pm$ 18	321 $\pm$ 23*
-2	457 $\pm$ 21	509 $\pm$ 28	396 $\pm$ 21*
-1	958 $\pm$ 194	874 $\pm$ 102	393 $\pm$ 25*
0	3520 $\pm$ 905	3965 $\pm$ 731	613 $\pm$ 52*
1	3692 $\pm$ 386*	5823 $\pm$ 809	1395 $\pm$ 88*
2	5326 $\pm$ 967*	8946 $\pm$ 1171	1192 $\pm$ 92*
3	6230 $\pm$ 835*	12052 $\pm$ 1535	1178 $\pm$ 96*
4	5379 $\pm$ 708*	14534 $\pm$ 1540	1409 $\pm$ 92*
5	7677 $\pm$ 825*	16182 $\pm$ 1581	1373 $\pm$ 113*

10

Effects of starting treatment with Test Compound once renal complications have become established.

15

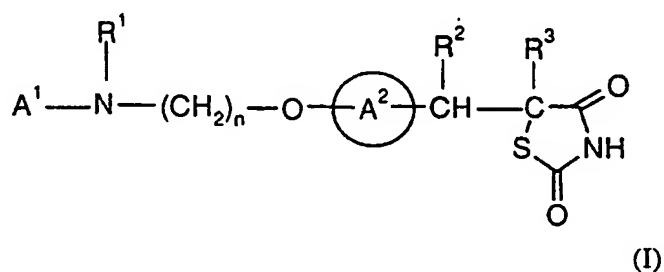
### URINARY N-ACETYL- $\beta$ -D-GLUCOSAMINIDASE ACTIVITY (mU/hour)

Treatment Duration (months)	Zucker fatty rats Test Compound (50 $\mu\text{mol}/\text{kg}$ of diet)	Zucker fatty rats Control (powdered chow)	Lean rats Control (powdered chow)
-4	5.4 $\pm$ 0.2	5.9 $\pm$ 0.3	4.4 $\pm$ 0.3*
-3	8.3 $\pm$ 0.5	9.1 $\pm$ 0.8	5.1 $\pm$ 0.5*
-2	9.5 $\pm$ 1.1	8.2 $\pm$ 1.5	6.5 $\pm$ 0.3
-1	7.8 $\pm$ 0.6	8.2 $\pm$ 1.0	5.2 $\pm$ 0.4*
0	7.6 $\pm$ 0.7	9.3 $\pm$ 0.5	6.9 $\pm$ 0.5*
1	7.6 $\pm$ 0.7	10.7 $\pm$ 0.4	7.6 $\pm$ 0.7*
2	5.7 $\pm$ 0.9	10.8 $\pm$ 1.7	7.4 $\pm$ 0.5
3	7.4 $\pm$ 0.9*	11.7 $\pm$ 0.7	7.1 $\pm$ 0.5*
4	5.4 $\pm$ 1.0*	11.8 $\pm$ 1.3	7.4 $\pm$ 0.5*
5	8.0 $\pm$ 1.1*	13.4 $\pm$ 0.8	7.7 $\pm$ 0.6*

20

### Claims:

1. A method for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease and microalbuminuria which method comprises the administration of an effective, non-toxic amount of an insulin sensitiser to a human or non-human mammal in need thereof.
2. A method according to claim 1, wherein the insulin sensitiser is a thiazolidinedione insulin sensitiser.
3. A method according to claim 1, wherein the insulin sensitiser is an acyclic insulin sensitiser.
4. A method according to claim 1, wherein the insulin sensitiser is a compound of formula (I):



or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof, and/or a pharmaceutically acceptable solvate thereof, wherein:

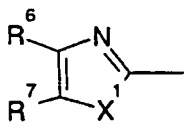
A<sup>1</sup> represents a substituted or unsubstituted aromatic heterocyclyl group;

- 25 R<sup>1</sup> represents a hydrogen atom, an alkyl group, an acyl group, an aralkyl group,  
wherein the aryl moiety may be substituted or unsubstituted, or a substituted or  
unsubstituted aryl group;  
R<sup>2</sup> and R<sup>3</sup> each represent hydrogen, or R<sup>2</sup> and R<sup>3</sup> together represent a bond;  
A<sup>2</sup> represents a benzene ring having in total up to five substituents; and  
30 n represents an integer in the range of from 2 to 6; to a human or non-human

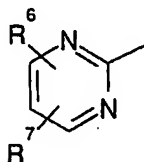
mammal in need thereof.

5. A method according to claim 4, wherein in the compound of formula (I) A<sup>1</sup> represents a moiety of formula (a), (b) or (c):

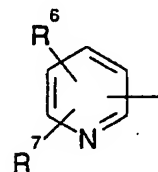
5



(a)



(b)

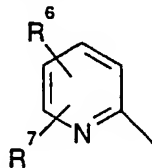


(c)

wherein:

R<sup>6</sup> and R<sup>7</sup> each independently represents a hydrogen or halogen atom, an alkyl or alkoxy group or a substituted or unsubstituted aryl group or when R<sup>6</sup> and R<sup>7</sup> are each attached to adjacent carbon atoms, then R<sup>6</sup> and R<sup>7</sup> together with the carbon atoms to which they are attached form a benzene ring wherein each carbon atom represented by R<sup>6</sup> and R<sup>7</sup> together is substituted or unsubstituted; and in the moiety of formula (a) X<sup>1</sup> represents oxygen or sulphur.

6. A method according to claim 5, wherein in the compound of formula (I) A<sup>1</sup> represents a moiety of the above defined formula (c).



(c')

wherein R<sup>6</sup> and R<sup>7</sup> are as defined in claim 5.

7. A method according to claim 1, wherein the insulin sensitiser is 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione or a tautomeric form thereof and/or a pharmaceutically acceptable salt thereof and/or a pharmaceutically acceptable solvate thereof.

8. A method according to claim 7, wherein the insulin sensitiser is a maleic

acid salt of 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione or a tautomeric form thereof and/or a pharmaceutically acceptable solvate thereof.

- 5    9.    The use of an insulin sensitiser for the manufacture of a medicament for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease and microalbuminuria.
- 10   10.   A pharmaceutical composition for the treatment and/or prophylaxis of renal diseases including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease and microalbuminuria which composition comprises an insulin sensitiser and a pharmaceutically acceptable carrier therefor.

15

## INTERNATIONAL SEARCH REPORT

 Inter:      al Application No  
 PCT/EP 95/00441

 A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 6    A61K31/00    A61K31/425    A61K31/44

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6    A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	J.CLIN.RES.DRUG DEV., vol. 8,no. 1, March 1994 pages 1-7, 'A perspective on the role of angiotensin-converting enzymes inhibitors in the treatment of hypertension' see page 4, paragraph 2 see page 6, references 25-28 ---	1-10
X	J.DIABET.COMPL., vol. 4,no. 2, - 1990 pages 75-8, 'Insulin sensitivity and blood lipids during antihypertensive treatment with special reference to ACE inhibition' see page 77, left column, line 53 - right column, line 35 --- -/--	1-10

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the international search

18 May 1995

Date of mailing of the international search report

06.06.95

Name and mailing address of the ISA

 European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+ 31-70) 340-3016

Authorized officer

Gerli, P

## INTERNATIONAL SEARCH REPORT

Intern. Patent Application No

PCT/EP 95/00441

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DRUGS, vol. 43,no. 4, 1992 pages 464-89, see page 464-5 summary see page 483 ---	1-10
Y	DIABETOLOGIA, vol. 36,no. supl.1, 1993 page A75. see abstract no. 285 ---	1-10
Y	WO,A,92 07850 (BEECHAM) 14 May 1992 see page 37; examples 7,8 see page 1, line 15 - line 30 ---	1-10
Y	EP,A,0 528 734 (ADIR ET CO.) 24 February 1993 see page 2, line 6 - line 23 see page 9, line 47 - line 52 see page 24, line 1 - line 15 see page 25, line 25 ---	1-10
Y	DATABASE WPI Derwent Publications Ltd., London, GB; AN 94-002284 & JP,A,05 310 719 (TERUMO CORP) , 22 November 1993 see abstract -----	1-10



# INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/EP 95/00441

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9207850	14-05-92	AU-B-	651388	21-07-94
		AU-A-	8732491	26-05-92
		EP-A-	0555264	18-08-93
		JP-T-	6502144	10-03-94
		NZ-A-	240378	23-12-93
-----				
EP-A-528734	24-02-93	FR-A-	2680512	26-02-93
		AU-B-	645709	20-01-94
		AU-A-	2110892	25-02-93
		JP-A-	5213913	24-08-93
		JP-B-	7030055	05-04-95
		NZ-A-	244021	27-01-95
		US-A-	5330999	19-07-94
		US-A-	5296605	22-03-94
		US-A-	5266582	30-11-93
ZA-A-	9206276	02-03-93		
-----				

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 95/00441

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 1-8 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.